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(71) Applicant: 000113470

Pola Chemical Industrial Company, Ltd.

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6-48 Yayoi-cho, Shizuoka-shi, Shizuoka-ken

(72) Inventor: Shigeharu Tanizawa

c/o Yokohama Research Laboratories  
Pola Chemical Industrial Company, Ltd.  
27-1 Takashimadai, Kanagawa-ku  
Yokohama-shi, Kanagawa-ken

(72) Inventor: Chihoko Suga

c/o Yokohama Research Laboratories  
Pola Chemical Industrial Company, Ltd.  
27-1 Takashimadai, Kanagawa-ku  
Yokohama-shi, Kanagawa-ken

(54) [Title of the Invention] A Method for the Determination of the anti-Stress Effects of  
Fragrances

(57) [Abstract] (With Corrections)

[Problem] The problem [that this invention sets out to solve] is to provide a method for the evaluation of the anti-stress effects of fragrances in correspondence to the type of stress.

[Method of solution] A method for the determination of the anti-stress effects of fragrances is provided in which psychological stress loading is effected in the presence of a fragrance, saliva is collected before and after stress loading, quantitative determination is made of the concentration of adrenocortical hormone in the saliva and the proportion of change is used as the index. By means of this invention, the anti-stress effects of fragrances can be determined objectively.

**[Claims]**

**[Claim 1]** A method for the determination of the anti-stress effects of fragrances in which psychological stress loading is effected in the presence of a fragrance, saliva is collected before and after stress loading, quantitative determination is made of the concentration of adrenocortical hormone in the saliva and the proportion of change is used as the index.

**[Claim 2]** A method as described in Claim 1 in which the adrenocortical hormone is cortisol.

**[Claim 3]** A method of determination as described in Claims 1 or 2 in which the method of stress loading is to perform numerical calculation within a fixed period for each problem or to perform numerical calculations with a warning being issued for mistakes such as difference in calculation.

**[Detailed Description of the Invention]****[0001]**

**[Field of industrial use]** This invention relates to a method for the determination of anti-stress effects of fragrances.

**[0002]**

**[Prior art]** It has long been suggested that there are various advantages associated with the use of perfumed cosmetic products. For example, such things are said as "they make the heart feel rich," "they put the heart at ease," "they round out the personality," "they give a person grace" and "they are good for the health." However, because the effects of perfume products on the body are extremely moderate, there are very few instances of determination of the actual action of perfume products on the body in numerical terms. For example, there is a study of elevation of globulin concentrations in body fluids by the fragrance of turnips. There are essentially no instances of corroboration of other effects. Although it is generally said the fragrances act to moderate stress, the only report of a case in which these points have been corroborated scientifically is to the effect that the quantity of cortisol that is secreted in the saliva when Kraepelin tests are performed in the presence of a fragrance is less than in the absence of a fragrance. There have been no studies whatsoever of relationships to the type of stress and or relationships to the type of fragrance. That is, it can be said that at present a technique for accurately evaluating the anti-stress effects of fragrances has not been fully obtained.

**[0003]** It is known, of course, that hormones, of which adrenocortical hormones are representative, regulate biological reactions in the body and that they are information transmitting substances for the purpose of intermodulation of these biological reactions. Although it is known that the concentration of this type of hormone in body fluids varies as a result of stimulation such as that of fragrances, it is still not known what sort of responses to stimulation occur. Further, although it is also known that concentrations of adrenocortical hormones are increased by stress loading, the relationship between the quality of stress and the concentration of adrenocortical hormones is not known.

**[0004]**

**[Problems the invention is intended to solve]** This invention was developed on the basis of these circumstances and provides a method for the evaluation of the anti-stress effects of fragrances corresponding to the type of stress.

**[0005]**

**[Means for solving the problems]** In view of these circumstances, the inventors conducted intensive and repeated studies for the purpose of finding means for scientific corroboration of the action of

perfume products, of which the effects of fragrances are representative, on life activities. As a result, this invention was perfected by discovering that the anti-stress effects of fragrances in the presence of psychological stress can be corroborated using changes in hormone levels in body fluids as the indicator. We shall now describe this invention in detail.

**[0006] (1) Method of evaluation of this invention**

In the method of evaluation of this invention, body fluids are collected before and after psychological stress loading in the presence of fragrances, quantitative determinations are made of hormones in body fluids and the anti-stress effect of the hormone is evaluated on the basis of changes in the hormone. The body fluids that can be used are blood, saliva and urine. However, because there is little effect on the subject and samples can be collected periodically at any desired time, the use of saliva is best. The next best method is collection of blood using an indwelling needle. However, a sufficient time is necessary after insertion of the needle. Adrenocortical hormones are desirable because they are highly related to stress. Of these, it is preferable to use cortisol as the indicator because quantitative determinations of it can be performed easily and it is found in higher concentrations than other adrenocortical hormones. There are no particular limitations on the method of quantitative determination as long as it is a method whereby determinations can be made to low concentrations. For example, the sandwich antibody method, the fluorescence labeled antibody method or the radioimmunoassay method can be used. Of these, the radioimmunoassay method is the most desirable because of its extremely high sensitivity. It has been suggested that adrenocortical hormones are highly related to stress. The inventors discovered that there is a close relationship between cortisol in saliva and stress as described subsequently. The action of fragrances on stress is evaluated scientifically by the method of evaluation of this invention.

**[0007] (2) The relationship between load of psychological stress and cortisol**

The following experiment was performed in order to ascertain the relationship between load of psychological stress and cortisol. Specifically, twenty subjects were assembled and they were asked to perform two-digit addition, subtraction, multiplication and division calculations with four seconds allowed for each problem. When they could not answer within the allowed time or when they made an error in calculation, they were warned with a buzzer sound. This procedure was carried out for 30 minutes. Saliva was collected before and after this 30 minute period and the concentration of cortisol in the saliva was found by radioimmunoassay as indicated in the mode of execution of this invention to be described subsequently. The ratio of increase of cortisol in the saliva was found by the formula, (concentration of cortisol before calculation)\*[sic] 100. Values were found on separate days when the allowance time was shortened to 3 seconds and when a period of 40 minutes was allotted for solving the problems. The results are shown in Table 1. From this table, it can be seen that the ratio of increase of cortisol rose in proportion to the load of psychological stress.

**[0008]**

**[Table 1]**

Conditions	Average ratio of increase of cortisol (%)
Allowance of 4 seconds, 30 minutes	43
Allowance of 3 seconds, 30 minutes	62
Allowance of 4 seconds, 40 minutes	56

**[0009]** When similar experiments were performed without presenting a warning with the buzzer sound, the results shown in Table 2 were obtained. From the table, it can be seen that the concentrations of cortisol did not increase as much as shown in Table 1. It was concluded that

cortisol does not increase solely because of calculation load. That is, it can be concluded that there is a close cause-and-effect relationship between increase of cortisol and psychological stress.

[0010]

[Table 2]

Conditions	Average ratio of increase of cortisol (%)
Allowance of 4 seconds, 30 minutes	11
Allowance of 3 seconds, 30 minutes	17
Allowance of 4 seconds, 40 minutes	13

[0011] A similar study was attempted using the Kraepelin test. The results are shown in Table 3. It was found that there was little psychological stress on the Kraepelin test.

[0012]

[Table 3]

Conditions	Average ratio of increase of cortisol (%)
30 minutes	9
45 minutes	10
60 minutes	10

[0013]

[Mode of execution of the invention] The mode of execution of this invention is comprised of the steps indicated below. Specifically, it is comprised of the following four steps.

- (Step 1) The subjects are kept at ease and saliva is collected.
- (Step 2) They are subjected to psychological stress load without presenting a fragrance.
- (Step 3) Saliva is collected.
- (Step 4) The cortisol concentration in the saliva is determined by the radioimmunoassay method and the ratio of increase of cortisol is calculated.

The steps are varied as follows and evaluations of fragrance are made. Specifically, it is comprised of the following five steps.

- (Step 1') The subjects are subjected to psychological stress.
- (Step 2') Saliva is collected.
- (Step 3') Fragrance is presented.
- (Step 4') Saliva is collected.
- (Step 5') Quantitative determination is made of the cortisol concentration in the saliva and the ratio of change in cortisol is calculated. Fragrance is evaluated on the basis of the change in the ratio of change over time.

We shall now describe these steps in detail.

[0014] (Step 1). At this stage, care must be taken to collect the saliva without putting a load on the subjects and collection must be performed naturally. After the subjects have entered the laboratory, music should be played and there must be a waiting period so that cortisol concentrations in the saliva will be at normal levels.

[0015] (Step 2). A desirable method is to induce high levels of psychological stress by physical stress. For example, the psychological stress of card games and a color reading process with a warning in which the subject is required to read the names of colors and state the names of colors in a table in which the colors are identified by color names different from their actual color names with the subject being warned with a buzzer sound when a mistake is made is employed. The preferable method is calculation load with a warning. Because this provides a high level of psychological stress load, the effect of the fragrance can be determined more accurately. The fragrance is presented immediately before this procedure. Presentation of the fragrance should be unobtrusive but to an extent at which it can be perceived. Specifically, on the order of  $0.01 \sim 1 \text{ mg/m}^3$  is desirable. There are no particular limitations on the method of presentation as long as it is an ordinary method; for example, methods using spraying and natural transpiration through the agency of volatilization promoting media such as fine thread wicks. Of these, spraying methods are preferable. In this step, care should be taken that the fragrance not be extinguished during the period of psychological stress loading.

[0016] (Step 3). In the collection of saliva, care must be taken so that the collection of saliva does not cause psychological stress. That is, it should be collected in as natural a way as possible. A tube may be left in the mouth so that the saliva can be suctioned off slowly. It is essential that the quantity collected be 0.3 to 0.6 ml.

[017] (Step 4). Determination of cortisol in the saliva may be performed by ordinary methods and may include methods using labeled antibodies and radioimmunoassay. From the standpoint of sensitivity, methods based on radioimmunoassay are preferable. There are commercial kits and they may be used. A kit of this type is, for example, Gamma Coro Cortisol [phonetic]\*, manufactured by the Baxter [phonetic] Company.

[18] The variations on the method in Step 1' to Step 5' may also be performed in accordance with these procedures.

[19]

[Examples]

Example 1

Cortisol decreasing action of fragrances

Twenty test subjects were subjected to the four second allowance 30-minute calculation load described above for 30 minutes in the presence of lavender, mint and skatole fragrances. After the procedure was completed, the degree to which the subjects experienced calmness in the presence of the respective fragrances was scored, with a score of 10 points indicating a feeling of calmness to an extremely great extent, a score of 0 indicating no feeling of calmness whatsoever, and a score of 1-10 indicating disturbance of calmness. In the control group, the procedure was performed without presenting fragrances. Saliva was collected before and after the procedure and quantitative determinations were made of cortisol using Gamma Cort Cortisol. Specifically, the saliva was frozen at  $-20^\circ\text{C}$  for 24 hours and was then restored to  $5^\circ\text{C}$ . It was centrifuged for 15 minutes at 3000 cpm and the supernatant was collected. The supernatant was stored at  $-20^\circ\text{C}$  until immediately before determination. One vial of the tracer solution in the kit was thoroughly mixed with 100 ml of the buffer solution in the kit to make a tracer buffer solution. Amounts of 200  $\mu\text{l}$  each of physiological saline solution as the blank and cortisol standard solution were introduced into the antibody tubes.

\* Translator's Note: Transliterated phonetically from the Japanese. As such, the spelling may differ from other transliterations.

Amounts of 1 ml each of tracer buffer solution were added to the tubes (T1 and T2) for use in total count determinations; caps were placed on the tubes and they were designated as total count tubes. These tubes were incubated at 37°C for 45 minutes. The solutions in them were removed and the radioactivity of all of the tubes was determined with a gamma ray counter. The radioactivity for the standard solution was plotted, a calibration curve was prepared and the concentration of cortisol in each sample was calculated from the calibration curve. The results are shown in Table 4. From this table, it can be seen that the concentrations of cortisol in the saliva were significantly decreased with mint and lavender. With skatole, the concentration of cortisol was somewhat elevated. These findings were well correlated with the order of calmness experienced by the panel.

[0020]

[Table 4]

Sample	Before Stress ( $\mu\text{g/dl}$ )	After Stress ( $\mu\text{g/dl}$ )	Average Score
Control	0.240	0.351	
Lavender	0.247	0.312	5.4
Mint	0.250	0.294	7.1
Skatole	0.248	0.376	-5.3

## [021] Example 2

Application of stress was effected by giving the subjects a table in which color names were described as colors different from the actual color names. In the first 15 minutes, they were asked to recite the names of the colors. In the final 15 minutes, they were asked to recite the names of the colors and were warned with a buzzer sound when they made a mistake. The changes in cortisol in the saliva were observed in the same way as in Example 1. The results are shown in Table 5. It was found that the anti-stress effects of fragrances on the application of stress in this way could be determined, although the stress was not as great as when calculations were performed with a warning as described above.

[0022]

[Table 5]

Sample	Before Stress ( $\mu\text{g/dl}$ )	After Stress ( $\mu\text{g/dl}$ )
Control	0.245	0.350
Lavender	0.241	0.309
Mint	0.239	0.286
Skatole	0.232	0.363

## [0023] Reference Example

Stress application was changed to the Kraepelin test and changes in cortisol in the saliva were observed in the same way as in Examples 1 and 2. The results are shown in Table 6. With the Kraepelin test, the effects were not as great as in Examples 1 and 2. This is because there is little psychological stress in the Kraepelin test. Consequently, the stress application method of this invention is more suitable than the Kraepelin test for determination of the anti-stress effects of fragrances.

[0024]

[Table 6]

Sample	After Stress ( $\mu\text{g/dl}$ )	Average Score ( $\mu\text{g/dl}$ )
Control	0.242	0.275
Lavender	0.233	0.251
Mint	0.244	0.264
Skatole	0.239	0.296

[025]

[Effect of the invention] Anti-stress effects of fragrances can be determined objectively by means of this invention.